Genetic analyses in patients with familial isolated hyperparathyroidism and hyperparathyroidism-jaw tumor syndrome

Short Title: Germline mutation of HRPT2 in patients with hyperparathyroidism

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Summary

Background

A subset of familial isolated primary hyperparathyroidism (FIHP) is a variant of hyperparathyroidism-jaw tumor syndrome (HPT-JT).

Aim/Patients and Methods

We investigated the involvement of the *HRPT2, MEN1*, and *CASR* genes in provisional 11 FIHP families and 2 HPT-JT families.

Results

Germline mutations of *HRPT2* were found in 2 of 11 FIHP families and 1 of 2 HPT-JT families. One FIHP family with parathyroid carcinoma and atypical adenomas, and another FIHP family with cystic parathyroid adenoma had novel frameshift mutations of 518-521del and 62-66del, respectively. In a patient with HPT-JT, a *de novo* germline mutation of 39delC was detected. Novel somatic *HRPT2* mutations of 70-73del and 95-102del were found in 2 of 5 parathyroid tumors in a family with 518-521del mutation. Biallelic inactivation of *HRPT2* by a combination of germline mutation and somatic mutation was confirmed in parathyroid tumors. The finding that 2 families diagnosed with FIHP carried *HRPT2* mutations suggests that they have occult HPT-JT. In the remaining 10 families, one family had a missense *MEN1* mutation. No mutations of *CASR* were detected.

Conclusion

Our results confirm the need to test for *HRPT2* in FIHP families, especially in

those with parathyroid carcinomas, atypical adenomas, or adenomas with cystic change.

Introduction

Primary hyperparathyroidism (PHPT) is usually a sporadic disorder, but in rare cases, it is part of hereditary endocrine tumor syndromes, namely multiple endocrine neoplasia types 1 and 2A (MEN1 and MEN2A), hyperparathyroidism-jaw tumor syndrome (HPT-JT), familial hypocalciuric hypercalcemia (FHH), or familial isolated hyperparathyroidism (FIHP).

MEN1 and MEN2A result from germline mutations of *MEN1* and *RET*, respectively.[1] Most cases of FHH result from a mutation of the calcium-sensing receptor gene (*CASR*).[2]

HPT-JT with an autosomal dominant mode of inheritance is predisposed to parathyroid tumors and jaw tumors. A relatively high frequency (15%) of parathyroid carcinoma was reported in patients with HPT-JT.[3] Furthermore, 30% of patients develop ossifying or cementifying fibroma of the mandible or maxilla. Renal lesions such as cysts and solid tumors, and uterine tumors were described.[4] The gene causing HPT-JT, *HRPT2*, is located on 1q25-q31.[5] Germline mutations of *HRPT2* have been identified in more than half of HPT-JT families.[4-10] In addition, somatic mutations of *HRPT2* were described in the majority of sporadic parathyroid carcinomas.[6,8,11]

FIHP is a diagnosis of exclusion, and may be due to incomplete expression of a syndrome or to other clinical entities. Germline mutations of *MEN1*,[12-15] *HRPT2*,[6,16,17] and *CASR*[3,18] genes were reported in a small group of FIHP families; however, mutations in the 5'-regulatory region or gross deletion of

HRPT2 have not been analyzed in FIHP families. [8,17,18]

In this study, we investigated the involvement of the *HRPT2, MEN1*, and *CASR* genes in Japanese families with 11 provisional FIHP and 2 HPT-JT.

Patients and Methods

Families

Eleven provisional FIHP (A to D, G to M) families and 2 HPT-JT (E and F) families were studied (Table 1). Data of biochemical examination, magnetic resonance (MR) or computed tomography (CT) scan of the pituitary and pancreas regions, and no cutaneous lesions such as lipomas and angiofibromas did not support the diagnosis of MEN1 in all FIHP families and HPT-JT families (Table 1). Physical examination revealed no jaw tumors in all FIHP families. In 8 of 11 families, absence of jaw tumors was confirmed by orthopantomography or CT (Table 1). The study was approved by our internal review board. Fully informed consent was obtained in accordance with institutional guidelines. The study was also conducted in accordance with the provisions of the Declaration of Helsinki.

Family A

A proband (II-1 in Fig. 1) died of parathyroid carcinoma. Sixteen years later, II-4 was found to have PHPT.[19] II-5 had an atypical parathyroid adenoma with cystic change. Ten years after the surgery of II-4, III-3 was found to have right urolithiasis and hydronephrosis. III-3 was found to have PHPT. The pathological diagnosis of parathyroid adenoma was made from a resected right-superior gland.

Family B

The proband (III-1) was found to have PHPT during hospitalization for the treatment of a left femoral fracture. A left-inferior parathyroid adenoma was resected. After the surgical operation, serum levels of calcium and PTH were within the normal range. Nine years after the initial operation, PHPT recurred. The resected right-inferior gland was diagnosed with adenoma with cystic change, while 2 other resected glands were normal. The paternal grandmother had multiple neck surgeries suggesting a possibility of parathyroidectomies, but her medical records could not be retrieved. Information on his father was not available.

Families C and D

In family C, the proband (I-1) was diagnosed with PHPT, which was also found in his daughter (II-1) and son (II-2). The diagnosis of parathyroid tumors in I-1 was carcinoma. [20] I-1 underwent repeated resection of metastatic tumors in the mediastinum; however, serum levels of calcium and PTH were elevated. In family D, a resected right-inferior parathyroid tumor of the proband (I-1) showed slight atypia and several capsular invasions, not vascular invasion. [21] Seven years later, the parathyroid tumor with lymph node metastasis was diagnosed as parathyroid carcinoma. Six months after the second surgery, he underwent *en bloc* resection of the tumor and trachea. He died of local recurrence of parathyroid carcinoma. Screening of family

members revealed that his son (II-1) had PHPT.

Family E

Four resected tumors of the proband (II-2) showed parathyroid hyperplasia. One year later, a cementifying fibroma in the left mandible was resected. [22] Her nephew (III-5) underwent total parathyroidectomy with transplantation. The parathyroid tumors in II-2 and III-5 did not have cystic features. The remaining 7 family members did not have PHPT, jaw tumors, or cystic kidney lesions. From these clinical data, the family was diagnosed as an HPT-JT family.

Family F

The proband (II-1 in Fig. 2) had noticed swelling of the right maxilla for 3 years, but its size had not changed. When he visited the family dentist for the treatment of caries, complete evaluation of the tumor was recommended. Biochemical screening revealed hypercalcemia. Subsequent CT of the neck suggested a left-superior parathyroid tumor. US did not show cysts or tumors in the kidney. A jaw tumor and a parathyroid tumor were resected and diagnosed as cement-ossifying fibroma and adenoma, respectively. [23] The parathyroid adenoma did not have cystic features. His younger sister underwent surgical operation due to Wilms' tumor at the age of 1. His parents and the younger sister showed normal levels of serum PTH and calcium.

From these clinical data, the patient was diagnosed with sporadic HPT-JT.

Families G to M

Clinical data on families G to M were shown in Table 1. In family M, II-1 and II-2 showed slightly elevated serum levels of gastrin, however, pancreatic tumors were not detected by CT scan. [24] The hypergastrinemia might have been due to gastric achlorhydria.

Preparation of DNA and RNA

Genomic DNA was isolated from leukocytes and parathyroid tumors by standard proteinase K-sodium dodecyl sulfate digestion and the phenol/chloroform method. Total RNA from tumors was prepared with ISOGEN (Nippon Gene, Tokyo, Japan).

Gene nucleotide sequence analysis

We searched for *HRPT2* mutations of by sequencing the entire coding region. The 17 coding exons of *HRPT2* were amplified as 16 different fragments with primers derived from the flanking intronic or untranslated regions. Furthermore, nucleotide sequences of the 5' regulatory region spanning 528 bp from the translation initiation site of *HRPT2* were analyzed. Somatic mutations of *HRPT2* in DNA from parathyroid tumors were analyzed. In addition, nucleotide sequencing of the coding region and exon-intron junctions

of the *MEN1* and *CASR* genes was analyzed. Primer sequences for the screening of *HRPT2*, *MEN1*, and *CASR* genes are available on request. PCR products were subjected to direct sequencing using an ABI PRISM BigDye™ terminators v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and analyzed on an ABI PRISM 3100 analyzer (Applied Biosystems).

Microsatellite analysis and loss of heterozygosity (LOH) analysis

Intragenic microsatellite markers located on introns 10 and 14 [11] in *HRPT2* were evaluated in DNA from leukocytes (families A to M) and from 5 parathyroid tumors (family A). Electrophoresis of 6-FAM-labeled products on an Applied Biosystems 3730xl DNA Analyzer was followed by pattern analysis using GeneMapper Software v3.5 (Applied Biosystems). LOH in parathyroid tumors was considered present when the signal intensity of one allele was reduced by more than 50% in comparison with the corresponding allele in normal DNA. In addition, information by direct sequencing of intragenic *HRPT2* polymorphisms and mutation was used for LOH analysis.

RNA analysis in parathyroid tumors

cDNA was synthesized from total RNA with random hexamers. cDNA was amplified with primers: 5'-GGGGAAGATGGCGGACGTGC-3' and 5'-GGTAGGTTCCCATAGTGTCAA-3'. The PCR amplified a 953 bp fragment containing a part of exon 1 through to a part of exon 7 of the *HRPT2*

gene. The PCR products were cloned into the pCR4 Blunt-TOPO vector with a Zero Blunt TOPO PCR Cloning Kit (Invitrogen Co., San Diego, CA). Each clone was sequenced using a sequencing kit.

Sodium bisulfite modification and bisulfite sequencing

Genomic DNA was modified by sodium bisulfite treatment and purified using a BisulFast Methylated DNA Detection Kit (TOYOBO, Osaka, Japan) according to the manufacturer's recommendations. The methylation status of the *HRPT2* promoter region was determined by bisulfite genomic sequencing as previously described. [25] Bisulfite-treated DNA was amplified with primers: 5'-TGTTGGGGATGGAAGTGTTGATTTATT -3' and 5'-ACAATCTCCTTCTTCTAAATATTATACTAT-3'. PCR products of 564 bp in size were cloned into the pCR4 Blunt-TOPO vector (Invitrogen Co.). Five clones each were sequenced using a sequencing kit.

Results

Nucleotide sequence analysis of *HRPT2*, *MEN1*, and *CASR* at the germline level

An *HRPT2* germline mutation of 518-521del was found in affected members of family A (II-3, II-4, II-5, and III-3) (Table 1 and Fig. 1). The mutation was not detected in an unaffected family member (II-6) or 50 normal Japanese individuals. An *HRPT2* germline mutation of 62-66del was detected in a

member of family B (Table 1). In a patient with PHPT and a jaw tumor (II-1 in Fig. 2), a germline mutation of 39delC was detected. The mutations of 518-521del, 62-66del, and 39delC resulted in a frameshift, leading to a premature stop codon.

In family F, the germline mutation of 39delC was not detected in his unaffected parents or his younger sister. Direct sequencing in exon 1 showed that the proband (II-1 in Fig. 2) and the proband's father (I-1 in Fig. 2) had a heterozygous nucleotide change of G to T at -10. Heterozygosity of G and T at -10 was detected in 1 subject among 50 normal Japanese individuals, suggesting a single nucleotide polymorphism (SNP). Subcloning and sequencing of PCR products in II-1 showed that clones with T at -10 and 39delC, and clones with G at -10 and wild type at 39 were separately obtained. Haplotype analysis in family F showed that the 39delC mutation is located on the paternal haplotype (Fig. 2), suggesting that the mutation occurred *de novo* in the father's spermatogenesis.

Although known polymorphisms of NCBI reference SNP ID number rs10737631 (A/C) in the 5' regulatory region and IVS2 + 28T/C/del4 were found, no mutations in *HRPT2* were identified in an HPT-JT family or in 9 other FIHP families (Table 1). Mutational analysis of *MEN1* showed a mutation of G225R (GGA to AGA) in family M. We further addressed germline mutations of *CASR*, however, none could be demonstrated (Table 1).

Nucleotide sequence analysis of *HRPT2* at the somatic level

HRPT2 screening in genomic DNA from 5 parathyroid tumors revealed that 2 adenomas had somatic mutations of 70-73del (an adenoma, II-4 in Fig. 1) and 95-102del (an atypical adenoma, II-5 in Fig. 1). Subcloning and sequencing of reverse transcription (RT)-PCR products showed that somatic mutations were located in the residual allele to the allele having the germline mutation. In an adenoma from II-4, we identified 10 clones. Among them, 2 clones contained only 518-521del and 7 clones contained only 70-73del. One clone contained a normal nucleotide sequence. In an atypical adenoma from II-5, 4 clones contained only 518-521del, and 3 clones contained only 95-102del among 8 clones selected. One clone contained a normal nucleotide sequence. In an atypical adenoma (II-4) and two adenomas (II-5 and III-3), somatic mutations in *HRPT2* were not detected.

LOH analysis in parathyroid tumors

Germline DNAs of patients in family A were heterozygous for at least one microsatellite marker of intron 10 or 14. Five tumors retained heterozygosity at the *HRPT2* locus. Results by direct sequencing of rs10737631, IVS2+28T/C/del4 or 518-521del in tumor DNA confirmed the retention of heterozygosity.

Methylation status of *HRPT2* promoter in parathyroid tumors

We analyzed the methylation status of the promoter region including 65 CpG sites in 5 parathyroid tumors and 4 corresponding leukocytes (Family A). No methylated CpG sites were found in DNA from tumors and leukocytes.

Discussion

We previously described family A as FIHP because of the following findings: 1) absence of jaw tumor and kidney lesions and 2) lack of LOH at 1q.[19] The majority of parathyroid tumors in HPT-JT patients are aggressive, occasionally recurrent adenomas, notable also for their cystic histology.[26] The development of 1 parathyroid carcinoma and 2 atypical adenomas suggested the possibility of HPT-JT in family A. 518del4 mutation was detected in patients with PHPT and a healthy mother (I-2 in Fig. 1) with normal serum levels of calcium and PTH. Nearly 10% to over 30% of mutation carriers of HPT-JT appear to remain clinically inert in adulthood.[3-4] This contrasts markedly with MEN1, which has an age-related penetrance of more than 98% by the age of 40.[27] A precise estimate of the age-related penetrance of *HRPT2* mutation requires information from a large number of families.

LOH at 1q24-q32 was identified in some, but not all HPT-JT-associated parathyroid tumors, suggestive of a tumor suppressor role for *HRPT2*.[28,29] Five parathyroid tumors in family A were negative for LOH at the *HRPT2* locus. The presence of somatic mutations on the residual allele to the germline mutation in 2 parathyroid tumors directly shows that the biallelic inactivation of *HRPT2* is associated with the disease. In parathyroid tumors with germline mutations of *HRPT2*, a second hit was observed in 7 of 12 tumors: LOH at *HRPT2* in 3 tumors and somatic mutations in 4 tumors.[6,10,11] LOH at *HRPT2* in HPT-JT-associated parathyroid tumors are not as frequent as

LOH at *MEN1* in MEN1-associated parathyroid tumors. [30,31] Transcriptional inactivation by hypermethylation of CpG islands containing gene promoter regions is one of the main mechanisms of tumor suppressor gene inactivation in tumors [32]; however, methylated CpG sites in the *HRPT2* promoter were not found in 5 parathyroid tumors from family A. The results suggested that promoter hypermethylation of *HRPT2* did not contribute to inactivation of the residual alleles. The mechanism of tumorigenesis in tumors without LOH or somatic mutations of *HRPT2* remains to be elucidated.

In family B, the proband with parathyroid adenoma with cystic change was considered to have sporadic parathyroid adenomas. Several patients with sporadic parathyroid carcinoma were found to have germline mutations of *HRPT2*, [6,8,11] suggesting an occult form of HPT-JT. Whether his grandmother had PHPT or not remains unclear; however, the identification of *HRPT2* mutation confirms that he can be classified as having HPT-JT. Our two families A and B as well as those reported by others [4,5,7,8,17] lacked the typical clinical manifestations of HPT-JT. From these results, genetic testing for *HRPT2* will be taken into consideration in FIHP families with parathyroid carcinomas, atypical adenomas, or cystic adenoma.

Although the father-derived *de novo* mutation of *RET* is a common phenomenon in MEN2B, [33] the *de novo* mutation of *HRPT2* has been reported in only one HPT-JT family showing mosaicism for L64P. [17] Haplotype analysis showed that the *de novo* mutation in family F was derived from the

father. The development of Wilms' tumor in 3 HPT-JT patients suggested that it might be a component of HPT-JT [34,35], however, the absence of *HRPT2* mutation in the younger sister with Wilms' tumor showed that Wilms' tumor was not a component of HPT-JT in family F.

The development of parathyroid carcinoma in FIHP families suggested HPT-JT; however, *HRPT2* mutations were not detected in families C and D. In addition, no germline mutations of *HRPT2* were detected in the remaining 7 FIHP families (families G to M). Although family E was diagnosed with HPT-JT based on clinical grounds, no germline mutations of *HRPT2* were detected. Indeed, Carpten *et al*. [5] reported that *HRPT2* sequencing fails to identify a mutation in about half of index patients from families with a full expression of HPT-JT and with proven genetic linkage to 1q24-q32. Mutations of the 5'-regulatory region of *HRPT2*, which have not been analyzed in other reports, were negative. So-far-unidentified mutations may also represent gross deletions or rearrangements in *HRPT2* that cannot be readily identified using direct sequencing; however, the retention of heterozygosity of polymorphic markers in families C to E (Table 1) suggested that the possibility of gross deletion of one allele, which was shown at the *MEN1* locus, [36,37] spanning the entire *HRPT2* gene was low. It is possible that other tumor suppressor genes may contribute to the development of parathyroid tumors in FIHP.

Missense *MEN1* mutation of G225R in family M was consistent with the

other report that FIHP families predominantly have missense mutations of *MEN1*.[14] The average percentage of *MEN1* mutations in FIHP families was reported to be 18%.[15] Lower frequency of *MEN1* mutations in our study might be due to exclusion of 2 families previously shown to carry *MEN1* mutations.

In conclusion, 2 families diagnosed with provisional FIHP were found to carry *HRPT2* germline mutations, and a father-derived *de novo HRPT2* germline mutation was found in a patient with HPT-JT. Our results confirm the need for testing for *HRPT2* in FIHP families, especially those with parathyroid carcinomas, atypical adenomas, or adenoma with cystic change.

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Figure legends

Figure 1. Pedigree of family A. Family members are indicated by generation (Roman numerals) and individuals (Arabic numerals). Individuals are represented as male (squares) and female (circles). The proband is indicated by an arrow. Closed symbols denote affected members. Open symbols denote unaffected members. Asterisks denote unaffected individuals confirmed by biochemical study and CT scan/orthopantomography. Sequencing of *HRPT2* showed a wild-type sequence (wt) or the presence of a mutation (mut). nd: germline mutations of *HRPT2* were not analyzed.

Figure 2. Paternal origin of *de novo* mutation of *HRPT2* in the family F. Genotypes in the *HRPT2* gene are shown for NCBI reference SNP ID number rs10737631 in the 5' regulatory region (rs10737631), a newly identified SNP at -10 from the translation initiation site (-10), an identified germline mutation (39delC) at 39 (39), a SNP at IVS2+28 (IVS2+28), a micosatellite marker in intron 10 (intron 10), and a micosatellite marker in intron 14 (intron 14). Wt denotes a normal sequence. The pedigree symbols are as described in Fig. 1.

Table 1. Clinical characteristics and HRPT2, MEN1, and CASR gene mutation analysis in FIHP and HPT-JT

Family	Patient number	Present age (yr)	Sex	PHPT, age at diagnosis (yr)	Serum calcium (mmol/L) normal range, 2.20-2.53	Serum intact PTH (ng/L) normal range, 10-65	Follow up after surgery years/Outcome	Jaw tumor, age at diagnosis (yr)	Kidney cystic lesions	Pituitary tumors, pancreatic tumors, lipomas, angiofibromas	Parathyroid tumors (no. and histology)	Numbers of family members screened	Germline mutations			Polymorphisms at *HRPT2*			
													HRPT2	*MEN1*	*CASR*	rs10737631	IVS2+28	intron 10	intron 14
A	II-1	died of parathyroid carcinima	M	27	4.29	C-PTH (μg/L) 15.5 normal range, less than 0.5		n.e.	No	No	carcinoma, metastasis to lungs	8	-	-	-	-	-	-	-
	II-4	44	F	34	3.99	822	11/hypoparathyroidism	No	No	No	1 atypical adenoma and 1 adenoma		518-521del	N	N	homo	homo	homo	hetero
	II-3	46	F	36	3.24	560	11/cured	No	No	No	1 adenoma		518-521del	N	N	hetero	hetero	hetero	hetero
	II-5	39	F	29	3.07	191	11/cured	No	No	No	1 cystic atypical adenoma		518-521del	N	N	hetero	hetero	hetero	hetero
	III-3	17	M	17	3.43	460	1/cured	No	No	No	1 adenoma		518-521del	N	N	homo	homo	hetero	hetero
B	III-1	40	M	28	3.15	198	2/cured	No	No	No	1 adenoma at 28 yrs, 1 cystic adenoma at 37 yrs	1	62-66del	N	N	homo	homo	hetero	homo
C	I-1	59	M	43	3.53	C-PTH (μg/L) 10.1	16/persistence	No	No	No	2 carcinomas at 43 yrs, 1 adenoma at 48 yrs, 1 carcinoma at 55 yrs , metastasis to mediastinum	3	-	-	-	-	-	-	-
	II-1	33	F	26	2.88	993	6/hypoparathyroidism	No	No	No	2 atypical adenomas and 1 hyperplasia		N	N	N	hetero	hetero	homo	hetero
	II-2	30	M	26	3.38	960	6/hypoparathyroidism	No	No	No	2 adenomas		N	N	N	hetero	hetero	homo	hetero
D	I-1	died of parathyroid carcinoma	M	57	3.25	C-PTH (μg/L) 2.5		n.e.	No	No	1 adenoma at 57 yrs, carcinima at 64 yrs (recurrence), carcinoma at 66 yrs (recurrence), metastasis to lymph nodes	4	-	-	-	-	-	-	-
	II-1	47	M	39	2.55	84	9/hypoparathyroidism	No	No	No	3 hyperplasia		N	N	N	hetero	homo	homo	homo
E	II-2	71	F	53	3.33	95	18/hypoparathyroidism	54	No	No	4 hyperplasia	9	N	N	N	homo	hetero	homo	homo
	III-5	30	M	19	2.95	79	11/hypoparathyroidism	No	No	No	1 adenoma		-	-	-	-	-	-	-
F	II-1	20	M	18	3.23	HS-PTH (ng/L) 3900 normal range,	2/cured	18	No	No	1 adenoma	4	39delC	-	-	homo	homo	hetero	hetero
G	II-2	58	F	55	2.9	178	4/cured	No	No	No	1 adenoma	2	N	N	N	hetero	hetero	homo	hetero
	II-1	71	F	65	2.85	132	6/cured	No	No	No	1 adenoma and 1 cyst		-	-	-	-	-	-	-
H	II-1	29	F	24	3.33	770	5/cured	n.e.	No	No	1 adenoma	2	N	N	N	homo	homo	homo	hetero
	I-2	50	F	40	-	-	10/cured	n.e.	No	No	1 adenoma		-	-	-	-	-	-	-
I	II-1	69	M	65	3.28	266	5/cured	n.e.	No	No	1 adenoma	2	N	N	N	hetero	hetero	hetero	hetero
	II-2	66	F	60	-	-	-	n.e.	-	-	1 adenoma		-	-	-	-	-	-	-
J	II-2	34	M	33	2.9	178	1/cured	No	No	No	4 hyperplasia	3	N	N	N	hetero	hetero	hetero	hetero
	II-1	39	M	38	2.98	105	1/cured	No	No	No	4 hyperplasia		-	-	-	-	-	-	-
	I-1	died of fulminant hepatitis	M	62	-	-		n.e.	-	-	-		-	-	-	-	-	-	-
K	I-2	74	F	69	2.63 ionized calcium (mmol/L) 1.28 normal range. 1.00-1.20	136	5/cured	No	No	No	1 adenoma	3	N	N	N	hetero	hetero	hetero	hetero
	II-1	48	F	44		58	4/cured	No	No	No	1 adenoma		-	-	-	-	-	-	-
L	II-6	55	F	54	2.83	140	1/cured	n.e.	No	No	1 adenoma	5	N	N	N	hetero	hetero	hetero	hetero
	II-3	65	F	62	2.85	99	3/cured	n.e.	No	No	1 adenoma		-	-	-	-	-	-	-
M	II-1	63	M	58	2.65	89.2	5/hypoparathyroism	No	No	No	2 hyperplasia	2	-	-	-	-	-	-	-
	II-2	57	F	52	2.8	116	6/hypoparathyroidism	No	No	No	4 hyperplasia		N	G225R	N	hetero	hetero	homo	hetero

M, male; F, female; -, Results not available or test not performed; n.e., Neither X-ray examination or CT scan was performed; N, normal nucleotide sequences; homo. homozygous: hetero, heterozygous.

Fig.1

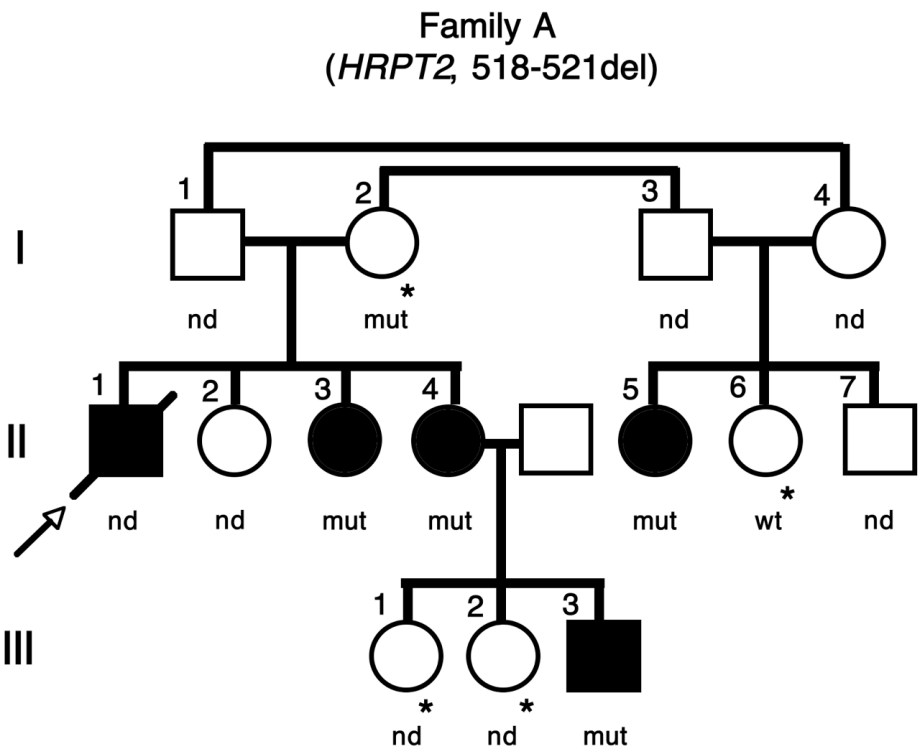
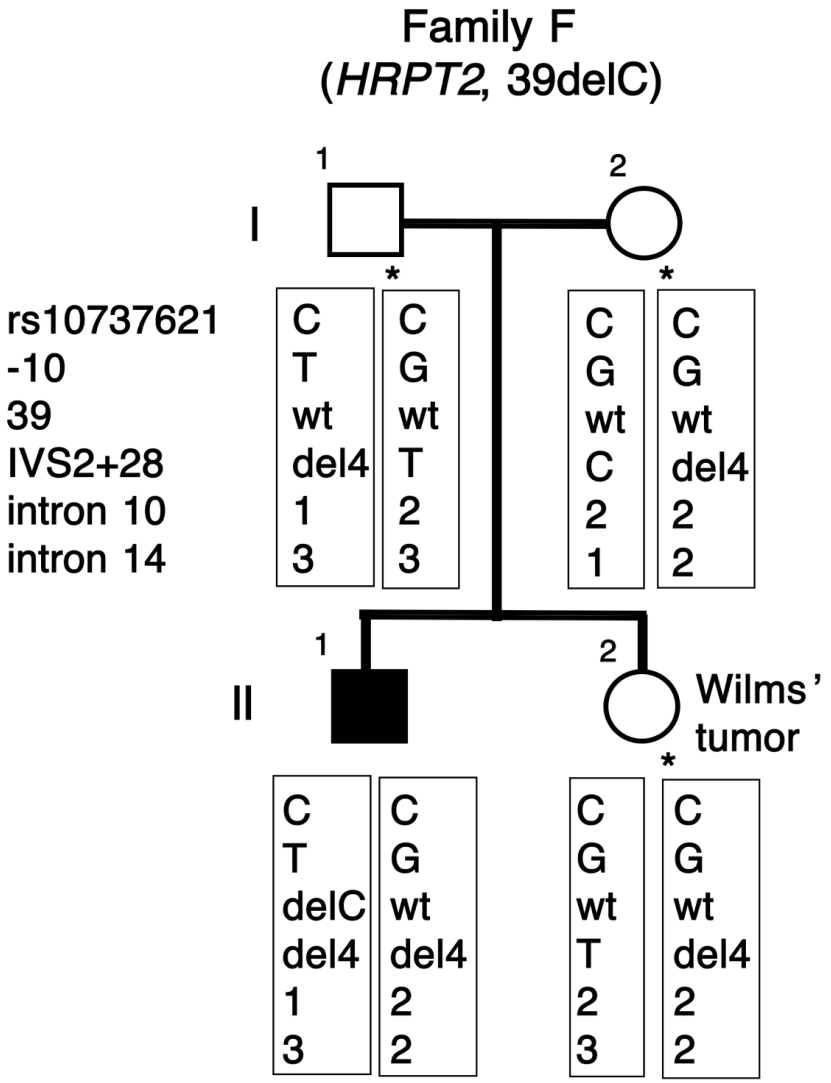

Family A
(*HRPT2*, 518-521del)
I
II
III
1
2
3
4
nd
mut
nd
nd
1
2
3
4
5
6
7
nd
nd
mut
mut
mut
wt
nd
1
2
3
nd
nd
mut

Fig. 2


Family F
(*HRPT2*, 39delC)
1
2
I
*
*
rs10737621
-10
39
IVS2+28
intron 10
intron 14
C
T
wt
del4
1
3
C
G
wt
T
2
3
C
G
wt
C
2
1
C
G
wt
del4
2
2
1
2
II
Wilms' tumor
*
C
T
delC
del4
1
3
C
G
wt
del4
2
2
C
G
wt
T
2
3
C
G
wt
del4
2
2